

REMARKS

This Reply is responsive to the Office Action dated February 23, 2010. Claims 49, 54-59, 61-63, and 68-79 were pending in the application at the time the Office Action was issued. Claims 68-77 are withdrawn as being directed to a non-elected invention and claims 49, 54-59, 61-63, 78, and 79 are under examination. Claims 57-59 and 78 have been cancelled. Claim 49, has been amended to incorporate the limitation of previously pending claim 59, namely that the claimed composition comprises chitosan and MPL as mucosal adjuvants. Applicant notes that claim 59 appears to be free of the prior art as it is not included in the rejections under §§102 or 103. Applicant also respectfully notes that these claim amendments do not raise new issues as the subject matter of currently amended claim 49 was pending and under examination in dependent claim 59. These amendments merely cancel claims and place rejected claims in better form for consideration on appeal, and are therefore in compliance with 37 C.F.R. §1.116. No prohibited new matter has been introduced by way of these amendments.

Upon entry of this amendment, claims 49, 54-56, 61-63, and 68-77, and 79 will be pending in the application with claims 49, 54-56, 61-63, and 79 under examination. Entry of the amendments and remarks submitted herein are respectfully requested.

I. Rejections under 35 U.S.C. §112, first paragraph

Claims 49, 54-59, 61-63, 78, and 79 stand rejected under 35 U.S.C. §112, first paragraph for allegedly failing to comply with the written description requirement. According to the Office Action, the specification does not provide adequate written description of dry powders containing immunogenic fragments and epitopes of protective antigen. The Examiner asserts that the definition of “anthrax peptide” in the specification includes immunogenic fragments and epitopes of anthrax antigens as well as full-length antigens, and thus concludes that recitation of “protective antigen in the claim necessarily includes homologs and fragments.” Page 3 of the Office Action. Based on the lack of knowledge and predictability in the art, the lack of corresponding homologs and lack of any characterized sequenced protein homologs or immunogenic fragment/epitopes, the Examiner asserts that one of ordinary skill in the art would not conclude that Applicant was in possession of the claimed genus of anthrax peptide variants formulated as a dry powder. Applicant respectfully disagrees and traverses the rejection.

As an initial matter, Applicant notes that claim 49 specifies that the dry powder composition comprises protective antigen not an “anthrax peptide.” Contrary to the Examiner’s assertion, protective antigen is not defined in the specification as including immunogenic fragments or homologs. The specification discloses that in preferred embodiments the composition comprises an anthrax peptide of LeTx and/or EdTx, such as PA, LF, EF, and/or immunogenic fragments thereof. See paragraph [009] (emphasis added). No where in the specification is protective antigen defined as including homologs and/or immunogenic fragments. Thus, recitation of protective antigen does not “necessarily include[] homologs and fragments.” Applicant respectfully notes that it is improper to read limitations from the specification into the claims. See MPEP §2111.01(II).

Nevertheless, Applicant submits that immunogenic fragments and epitopes of protective antigen were well known in the art at the time the invention was made. For instance, as evidenced by Flick-Smith *et al.* (Infection and Immunity, Vol. 70: 1653-1656, 2002; submitted herewith), fragments containing various domains of protective antigen were shown to be immunogenic and protect mice against an anthrax challenge. See Table 1 and pages 1654-1655. Furthermore, the crystal structure of protective antigen was known in the art as well as the function of each of the identified domains of protective antigen. See page 1653, left-hand column of Flick-Smith. The specification provides a description of dry powder compositions comprising protective antigen as well as a working example demonstrating that such dry powder compositions ameliorate or prevent at least one symptom of anthrax disease. Given the existing knowledge in the art of how to prepare immunogenic fragments of protective antigen that protect against anthrax challenge, one of ordinary skill in the art would know how to make immunogenic fragments of protective antigen and use of such immunogenic fragments in the compositions would be considered to be equivalent.

With respect to formulation of the immunogenic fragments as dry powder compositions, the Examiner asserts that “[a]pplicants are claiming in broad terms a dry powder mucosal vaccine but do not teach how to make such or do not reference any particular means in the art to arrive at such.” See page 4 of Action. Later in the Office Action, however, the Examiner also argues that “[f]ormulation as a dry powder for long term preservation and/or administration is routine in the pharmaceutical arts.” Page 7 of Action. The above comments appear to be

contradictory and the Examiner's position is therefore unclear. Applicant respectfully requests clarification.

In any case, Applicant need only provide a description of the claimed invention in sufficient detail to convey to one skilled in the art that the inventor had possession of the claimed invention as of the application filing date. See MPEP §2163(I). Accordingly, Applicant submits that the specification satisfies the written description requirement and the rejection of the claims under 35 U.S.C. §112, first paragraph should be withdrawn.

II. Rejections under 35 U.S.C. §102(a)

Claims 49, 57, 58, 61-63, and 79 stand rejected under 35 U.S.C. §102(a) as being anticipated by a literature publication to Miksztra *et al.* (Journal of Infectious Diseases, Vol. 191: 278-288, January 15, 2005). Miksztra *et al.* allegedly disclose dry powder formulations of recombinant protective antigen, CpG, trehalose, and chitosan loaded into capsules for intranasal delivery with a device. The Examiner asserts that the Declaration under 37 CFR §1.131 submitted by Applicant is insufficient to antedate Miksztra *et al.* because it allegedly fails to establish an earlier reduction to practice before the critical date. According to the Office Action, Applicant is required to show the same species (*e.g.* CpG) as the reference to successfully antedate the reference where the reference is applied against a genus claim. Applicant respectfully traverses the rejection.

Applicant notes that a Declaration under §1.131 is not insufficient simply because it does not show the identical disclosure of the cited art reference. See MPEP §715.02. According to the MPEP, "[i]f the affidavit contains facts showing a completion of the invention commensurate with the extent of the invention as claimed is shown in the reference or activity, the affidavit or declaration is sufficient, whether or not it is a showing of the identical disclosure of the reference or the identical subject matter involved in the activity." MPEP §715.02 (citing *In re Wakefield*, 422 F.2d 897 (CCPA 1970)).

Nevertheless, solely to expedite prosecution of the application, claim 49 has been amended to specify that the composition comprises MPL and chitosan as mucosal adjuvants. This subject matter was previously pending in dependent claim 59. Miksztra *et al.* do not disclose a dry powder composition comprising protective antigen, chitosan and MPL. Therefore, Miksztra *et al.* fail to disclose all the limitations of the claims and the rejection of the claims

under 35 U.S.C. §102(a) should be withdrawn. Applicant reserves the right to pursue the broader subject matter in a continuation application.

III. Rejections under 35 U.S.C. §103(a)

Claims 49, 54-57, 61-63, 78, and 79 stand rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Publication No. 2006/0134143 to Schneerson. The Examiner maintains her position that it would have been *prima facie* obvious to one of skill in the art to combine the recombinant protective antigen conjugated to PGA peptide with MPL and formulate the composition as a dry powder and administer to a mucosal surface as allegedly suggested by Schneerson. Applicant respectfully traverses the rejection as to the amended claim above.

Claim 49 has been amended to incorporate the limitations of claim 59 and is now directed to a dry powder composition comprising protective antigen, MPL and chitosan. Schneerson does not teach or suggest such a composition, and indeed, previously pending claim 59 was not included in the rejection. Accordingly, this rejection appears to be moot and Applicant respectfully requests that it now be withdrawn.

Claims 49, 54-57, 61-63, 78, and 79 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Schneerson in view of an abstract by Alpar *et al.* As discussed above, claim 49 has been amended to incorporate the limitation of previously pending claim 59, namely that the composition comprises protective antigen, MPL, and chitosan. Applicant notes that again, claim 59 is not included in the current rejection. Therefore, it appears that this rejection is now moot.

Indeed, although an express suggestion in the references themselves is not required to establish a *prima facie* case of obvious, where there is a broad genus of compounds or several variables disclosed, there must be some motivation or suggestion for the skilled artisan to select the particular species or subgenus from the broad genus disclosed in the reference. See MPEP §2144.08 (II)(A)(4). “The fact that a claimed species of subgenus is encompassed by a prior art genus is not sufficient by itself to establish a *prima facie* case of obviousness.” MPEP §2144.08 (II)(citing *In re Baird*, 16 F.3d 380 (Fed. Cir. 1994)). In the instant case, Schneerson provides a boilerplate, laundry list disclosure of carriers for conjugation to the PGA peptide, additives for pharmaceutical compositions, including a laundry list of adjuvants, and various types of formulations, including liquids, gels, powders, pastes, films, microspheres, and viscous solutions.

In reading Schneerson, the skilled artisan would have no way of knowing that use of MPL, in particular, in a dry powder formulation would convey a protective immune response against anthrax. Alpar *et al.* do not make up for the deficiency of Schneerson. There is no teaching in Alpar *et al.* that would suggest the claimed composition.

Applicant has surprisingly found that the claimed composition provides protective, sterile immunity against Anthrax challenge. See paragraphs [0152] and [0153] of the specification. Such compositions are not taught or suggested by the prior art and thus, the claimed invention is nonobvious over the cited references. Applicant respectfully submits that the rejection may now be withdrawn.

IV. Claim Objections Rejection under 35 U.S.C. §112, second paragraph

Claim 78 is objected to for allegedly being of improper dependent form. According to the Office Action, claim 78 does not further limit the subject matter of the previous claim from which it depends. Claim 78 has also been rejected under 35 U.S.C. §112, second paragraph for allegedly being indefinite because it is unclear whether Applicant is claiming a dry powder composition or a liquid composition.

Without agreeing with either rejection, claim 78 has been cancelled, thus rendering both the objection and the rejection of this claim moot.

CONCLUSION

This reply is fully responsive to the Office Action dated February 23, 2010. Applicant respectfully requests favorable reconsideration and allowance of the pending claims in view of the foregoing amendments and remarks.

Except for issue fees payable under 37 CFR §1.18, the commissioner is hereby authorized by this paper to charge any additional fees during the pendency of this application including fees due under 37 CFR §1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-1283. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 CFR §1.136(a)(3).

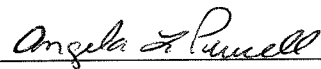
If the Examiner has any further questions relating to this Reply or to the application in general, she is respectfully requested to contact the undersigned by telephone so that allowance of the present application may be expedited.

Dated: July 23, 2010

Respectfully submitted,
COOLEY LLP

CUSTOMER NO. 58249

By:


Angela Purcell
Reg. No. 60,642

Cooley LLP
ATTN: Patent Group
777 6th Street, NW, Suite 1100
Washington, DC 20001
Tel: (202) 842-7859
Fax: (202) 842-7899

A Recombinant Carboxy-Terminal Domain of the Protective Antigen of *Bacillus anthracis* Protects Mice against Anthrax Infection

Helen C. Flick-Smith, Nicola J. Walker, Paula Gibson, Helen Bullifent, Sarah Hayward, Julie Miller, Richard W. Titball, and E. Diane Williamson*

Dstl, Chemical and Biological Sciences, Porton Down, Salisbury, Wiltshire SP4 0JQ, United Kingdom

Received 25 July 2001/Returned for modification 26 September 2001/Accepted 12 November 2001

The immunogenicity and protective efficacy of overlapping regions of the protective antigen (PA) polypeptide, cloned and expressed as glutathione S-transferase fusion proteins, have been assessed. Results show that protection can be attributed to individual domains and imply that it is domain 4 which contains the dominant protective epitopes of PA.

Protective antigen (PA) is the dominant antigen in both natural and vaccine-induced immunity to anthrax infection. It is also essential for host cell intoxication in combination with either lethal factor (LF) or edema factor (EF), producing lethal toxin or edema toxin, respectively (6), as it contains the host cell receptor binding site (5) and facilitates the entry of the toxin complex into the host cell. The crystal structure of native PA has been elucidated (15) and shows that PA consists of four distinct and functionally independent domains. Domain 1 is divided into domains 1a, comprising amino acids 1 to 167, and 1b, comprising amino acids 168 to 258; domain 2 comprises amino acids 259 to 487; domain 3 comprises amino acids 488 to 595; and domain 4 comprises amino acids 596 to 735. Cell intoxication is thought to occur when full-length PA (PA83) binds to the cell surface receptor via domain 4, which contains the host cell receptor binding site (10). On binding to the host cell receptor, the N-terminal amino acids (1 to 167, i.e., domain 1a) of domain 1, which contains a furin protease cleavage site (8), are cleaved off, exposing the LF or EF binding site located in domain 1b and the adjacent domain 3 (15). Domains 2 and 3 then form part of a heptameric pore on the cell surface (13, 14), the LF or EF binds to its receptor, and the whole toxin complex undergoes receptor-mediated endocytosis into the cell. After acidification of the endosome, the toxin is translocated into the cell cytosol, where it exerts its cytotoxic effect (7). Therefore, inhibition of the binding and entry of the toxin complex, particularly lethal toxin, into the host cell is clearly important for the prevention of infection.

Anthrax occurs in three main forms: cutaneous, gastrointestinal, and pulmonary (16). If diagnosed early enough, infection can be treated with antibiotics, but symptoms are not always apparent in time for antibiotic treatment to be effective, so vaccination is essential to protect individuals who are at risk of exposure. The current anthrax vaccine licensed in the United Kingdom is an alum-precipitated filtrate of *Bacillus anthracis* strain Sterne cultures grown to maximize the PA content (17). The production of this vaccine requires containment facilities,

and the vaccine itself varies in PA content from batch to batch. There are also problems of transient reactogenicity due to the presence of small amounts of LF and EF and other bacterial components and a limited duration of protection requiring frequent boosts for continued immunity. These problems could be resolved by refining the vaccine components and enriching the vaccine with respect to the key protective component, PA. It is known that antibodies to PA are essential for immunity to anthrax infection (11), but it is not known if the whole PA polypeptide is required to stimulate an effective immune response or whether certain subunits of PA would be equally effective immunogens. To investigate this, a panel of recombinant PA (rPA) domain proteins has been produced and used to immunize mice to assess their immunogenicity and protective efficacy against anthrax spore challenge and also to ascertain if the presence of any one domain is most critical for protection.

Cloning and protein expression. DNA encoding the PA domains, which comprise amino acids 1 to 258, 168 to 487, 1 to 487, 168 to 595, 1 to 595, 259 to 735, 488 to 735, 596 to 735, and 1 to 735 (fusion proteins GST1, GST1b-2, GST1-2, GST1b-3, GST1-3, GST2-4, GST3-4, GST4, and GST1-4, respectively), was PCR amplified from *B. anthracis* strain Sterne DNA and cloned into the *Xho*I and *Bam*HI sites of the expression vector pGEX-6-P3 (Amersham-Pharmacia) downstream of and in frame with the *lac* promoter. Proteins produced by this system were expressed as fusion proteins with an N-terminal glutathione S-transferase (GST) protein. Initial extraction of the fusion proteins indicated that they were produced as inclusion bodies. These were solubilized by using 8 M urea and renatured by dialysis against an arginine buffer to stabilize the protein upon refolding. Successful refolding of the fusion proteins allowed them to be purified on a glutathione Sepharose CL-4B affinity column (Amersham-Pharmacia). However, fusion protein GST1b-2 (amino acid residues 168 to 487) could not be eluted from this column and was therefore purified by ion-exchange chromatography. The yields of the fusion proteins varied between 1 and 43 mg liter of culture⁻¹. To check that the fusion proteins were of the expected molecular weights and that they could be recognized by antibodies to PA, the fusion proteins were run on sodium dodecyl sulfate (SDS)–10 to 15% polyacrylamide gels (PhastGel; Amersham-

* Corresponding author. Mailing address: Dstl, Chemical and Biological Sciences, Porton Down, Salisbury, Wiltshire SP4 0JQ, United Kingdom. Phone: 01980 613895. Fax: 01980 614307. E-mail: dewilliamson@dstl.gov.uk.

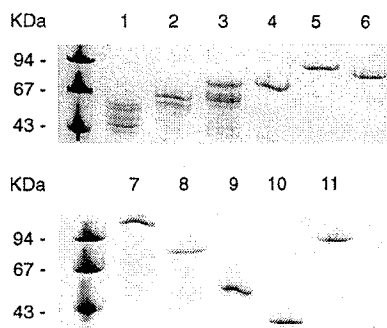


FIG. 1. SDS-PAGE analysis of GST fusion proteins. Lane 1, GST1; lane 2, GST1b-2; lane 3, GST1-2; lane 4, GST1b-3; lane 5, GST1-3; lane 6, rPA; lane 7, GST1-4; lane 8, GST2-4; lane 9, GST3-4; lane 10, GST4; lane 11, rPA.

Pharmacia). Protein bands were detected either by staining with PhastGel Blue R or, after electrophoretic transfer onto polyvinylidene difluoride membranes (Millipore), by using mouse anti-rPA sera. Analysis of the fusion proteins by SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 1) and Western blotting (Fig. 2) showed protein bands of the expected sizes that specifically bound anti-rPA antisera, showing that the GST protein tag did not interfere with PA epitope recognition in vitro. Some degradation was apparent in all of the fusion proteins investigated, showing similarity with rPA expressed in *Bacillus subtilis*. The rPA-truncated proteins GST1, GST1b-2, and GST1-2 were particularly susceptible to degradation in the absence of domain 3. This has similarly been reported for rPA constructs that contained mutations in domain 3 and that could not be purified from *B. anthracis* culture supernatants (3), suggesting that domain 3 may stabilize domains 1 and 2.

Immunization and challenge. Female A/J mice (Harlan Olac, Blackthorn, United Kingdom) were immunized intra-

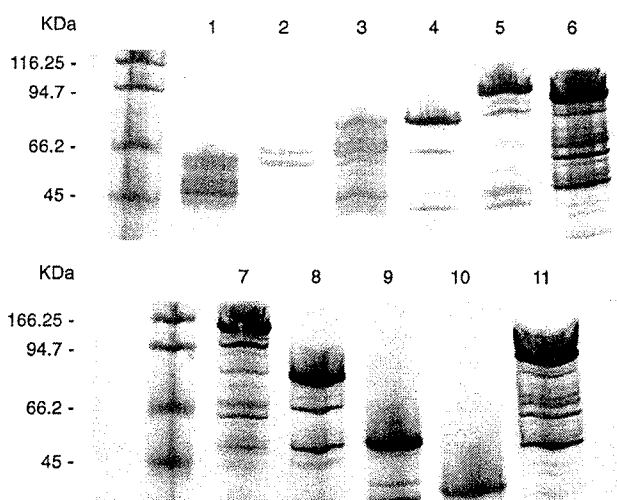


FIG. 2. Western blot analysis of fusion proteins. Proteins were detected by using mouse anti-rPA antisera. Lane 1, GST1; lane 2, GST1b-2; lane 3, GST1-2; lane 4, GST1b-3; lane 5, GST1-3; lane 6, rPA; lane 7, GST1-4; lane 8, GST2-4; lane 9, GST3-4; lane 10, GST4; lane 11, rPA.

muscularly (i.m.) with 10 μ g of protein adsorbed to a 20% (vol/vol) solution of 1.3% Alhydrogel (HCI Biosector, Fredrikssund, Denmark) on days 1 and 28 of the study. We also included groups of mice that were immunized with rPA (expressed and purified from *B. subtilis* [12]), with recombinant GST control protein, or with fusion proteins comprising domains 1, 4, and 1 to 4 which had had the GST tag removed by incubation with PreScission Protease (16 h at 4°C; Amersham-Pharmacia) and removal of the GST on a glutathione Sepharose column. Blood samples from mice were collected 37 days after primary immunization for serum antibody analysis by enzyme-linked immunosorbent assay. Mice were challenged intraperitoneally with either 10^5 or 10^6 spores of the *B. anthracis* STI strain (equivalent to 10^2 or 10^3 minimum lethal doses [MLDs] [1], respectively) on day 70 of the immunization regimen and were monitored for 14 days postchallenge to determine their protected status. Humane treatment end points were strictly observed so that any animal displaying a predetermined set of clinical signs, which together indicated that it had a lethal infection, was culled.

All of the fusion proteins were immunogenic and stimulated mean serum anti-rPA immunoglobulin G (IgG) concentrations in the A/J mice ranging from 6 μ g ml $^{-1}$ for the GST1b-2 fusion protein-immunized group to 1,488 μ g ml $^{-1}$ for the GST1-4 fusion protein-immunized group (Fig. 3). The control mice, immunized with GST only, had no detectable antibodies to rPA. The predominant subclass of IgG stimulated by immunization with any of the fusion proteins was IgG1, followed by IgG2a and IgG2b in lesser amounts and by IgG3, of which there was no detectable amount. The IgG subclass data show a strong bias to a Th2 type of response after immunization with either the fusion proteins or rPA in the presence of Alhydrogel. This has previously been noted after rPA immunization in the presence of Alhydrogel (20) and shows that the qualitative immune response stimulated after immunization with complete or partial domains of rPA is the same as that stimulated by the whole rPA, irrespective of whether protection is conferred.

Mice were challenged 42 days after booster immunization, and the numbers of mice which survived 14 days postchallenge are shown in Table 1. At the lower challenge level of 10^2 MLDs, mice in the GST1-2-, GST4-, and cleaved 4-immunized groups were all fully protected, but some breakthrough in protection for those in the groups immunized with GST1, cleaved 1, GST1b-2, GST1b-3, and GST1-3 was observed despite their having functionally significant anti-rPA titers (with the exception of the GST1b-2-immunized group). The mice in these groups that died had a mean time to death (MTTD) of 4.5 ± 0.2 days, which was not significantly different from that of the GST control-immunized group, in which all mice died with an MTTD of 4 ± 0.4 days. This suggests that the immune response had not been appropriately primed by these proteins to achieve resistance to the infection. As has been shown in other studies, for mice and guinea pigs (9, 18), there is no precise correlation between antibody titer to PA and protection against challenge, although a certain threshold of antibody titer may be required for protection (4), suggesting that the stimulation of cell-mediated components of the immune response is also necessary. GST1, GST1b-2, and GST1-2 were the least stable fusion proteins produced, as shown by the

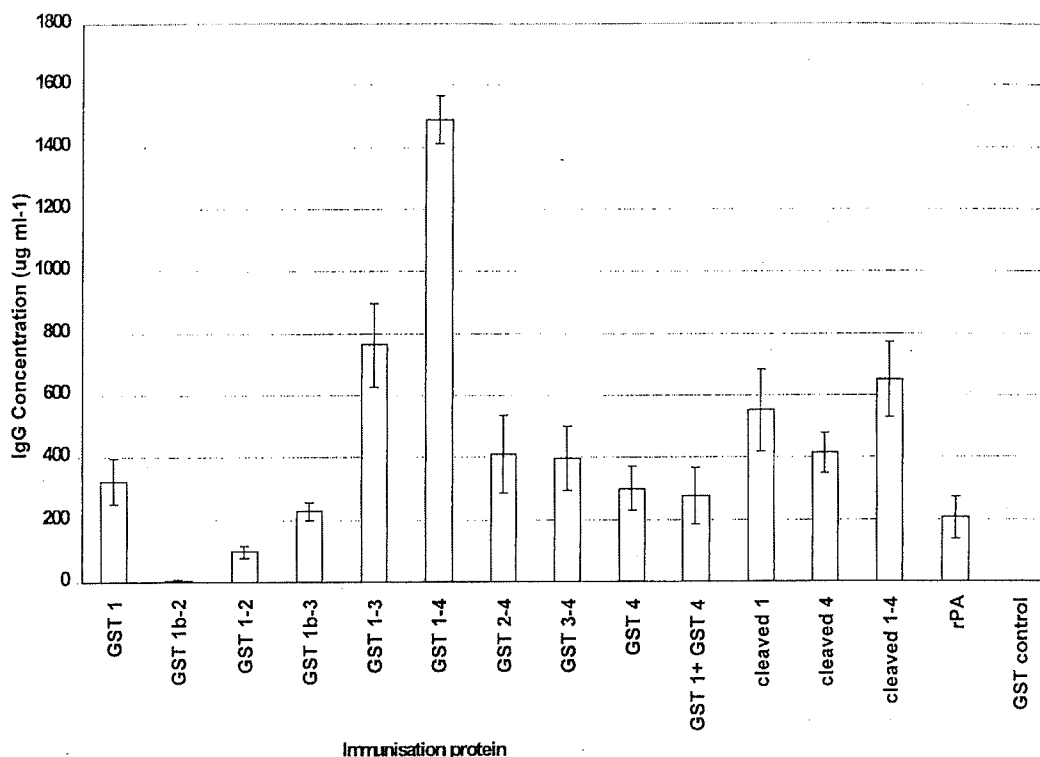


FIG. 3. Anti-rPA IgG concentrations 37 days after primary immunization in A/J mice immunized i.m. on days 1 and 28 with either 10 µg of fusion protein or 10 µg of domain protein cleaved from GST. Results shown are means \pm standard errors of the mean for samples taken from five mice per treatment group.

SDS-PAGE and Western blotting results. This instability was possibly due to the proteins' greater susceptibility to degradation in the absence of domain 3 and may have resulted in the loss of protective epitopes. The structural conformation of the proteins may also be important for stimulating a protective

immune response. The removal of domain 1a from the fusion proteins yielded both reduced antibody titers and lower levels of protection against challenge than were found for the intact counterparts GST1-2 and GST1-3. Similarly, mice immunized with GST1 alone were partially protected against challenge, but when this immunization was combined with that involving domain 2, as in the GST1-2 fusion protein, full protection was seen at the 10^2 -MLD challenge level. However, the immune response stimulated by immunization with the GST1-2 fusion protein was insufficient to provide full protection against the higher, 10^3 -MLD challenge level, which again could be due to the loss of protective epitopes caused by degradation of the protein.

The mice in the groups challenged with 10^3 MLDs of STI spores were all fully protected, except for those in the GST1-, GST1-2-, and cleaved 1-immunized groups, for which there was some breakthrough in protection, and those in the control group immunized with GST only, which succumbed to infection with an MTTD of 2.4 ± 0.2 days. All mice in the groups immunized with fusion proteins containing domain 4 were fully protected against challenge with 10^3 MLDs of STI spores (Table 1). Brossier et al. showed a decrease in protection for mice immunized with a mutated strain of *B. anthracis* that expressed PA without domain 4 (2), and this was confirmed in this study, where immunization with GST1-3 resulted in a breakthrough in protection despite high antibody titers.

These data indicate that domain 4 contains the dominant

TABLE 1. Survival of A/J mice^a

Fusion protein	No. of survivors/no. challenged (%) at:	
	10^2 MLDs ^b	10^3 MLDs
GST1	3/5 (60)	1/5 (20)
GST1b-2	1/5 (20)	ND ^c
GST1-2	5/5 (100)	3/5 (60)
GST1b-3	3/5 (60)	ND
GST1-3	4/5 (80)	ND
GST1-4	ND	5/5 (100)
GST2-4	ND	5/5 (100)
GST3-4	ND	5/5 (100)
GST4	5/5 (100)	5/5 (100)
GST1 + GST4	ND	5/5 (100)
Cleaved 1	1/5 (20)	2/5 (40)
Cleaved 4	5/5 (100)	5/5 (100)
Cleaved 1-4	ND	5/5 (100)
rPA	ND	4/4 (100)
Control	0/5 (0)	0/5 (0)

^a Mice were immunized i.m. on days 1 and 28 with either 10 µg of fusion protein or 10 µg of domain protein cleaved from GST and observed for 14 days postchallenge with STI spores.

^b 1 MLD = approximately 10^3 STI spores (1).

^c ND, not done.

protective epitopes of PA. Domain 4 represents the 139 amino acids of the carboxy terminus of the PA polypeptide. It contains the host cell receptor binding region (10), which has been identified as being in and near a small loop located between amino acid residues 679 and 693 (19), and it is therefore essential for host cell intoxication, as previous studies have demonstrated that expressed forms of PA containing mutations (19) or deletions (3) in the region of domain 4 are non-toxic. The crystal structure of PA shows domain 4, and in particular a 19-amino-acid loop of the domain (703 to 722), to be more exposed than the other three domains, which are closely associated with each other (15). This structural arrangement may make the epitopes in domain 4 the most prominent for recognition by immune effector cells.

This investigation has further elucidated the role of PA in the stimulation of a protective immune response, demonstrating that protection against anthrax infection can be attributed to individual domains of PA. Work is continuing to explore the immune mechanisms stimulated by immunization with rPA domains, to investigate immunoreactivity to the domains as surrogate markers of efficacy, and to examine the utility of these domains as novel vaccine candidates.

We thank E. Waters, C. Redmond, and D. Rogers for their excellent technical assistance.

REFERENCES

1. Beedham, R. J. 1999. M.Phil. thesis. The Open University, Milton Keynes, United Kingdom.
2. Brossier, F., M. Weber-Levy, M. Mock, and J.-C. Sirard. 2000. Role of toxin functional domains in anthrax pathogenesis. *Infect. Immun.* 68:1781-1786.
3. Brossier, F., J.-C. Sirard, C. Guidi-Rontani, E. Dufrot, and M. Mock. 1999. Functional analysis of the carboxy-terminal domain of *Bacillus anthracis* protective antigen. *Infect. Immun.* 67:964-967.
4. Cohen, S., I. Mendelson, Z. Altboum, D. Kobiler, E. Elhanany, T. Bino, M. Leitner, I. Inbar, H. Rosenberg, Y. Gozes, R. Barak, M. Fisher, C. Kronman, B. Velan, and A. Shafferman. 2000. Attenuated nontoxigenic and nonencapsulated recombinant *Bacillus anthracis* spore vaccines protect against anthrax. *Infect. Immun.* 68:4549-4558.
5. Escuyer, V., and R. J. Collier. 1991. Anthrax protective antigen interacts with a specific receptor on the surface of CHO-K1 cells. *Infect. Immun.* 59:3381-3386.
6. Ezzell, J. W., B. E. Ivins, and S. H. Leppla. 1984. Immunoelectrophoretic analysis, toxicity, and kinetics of in vitro production of the protective antigen and lethal factor components of *Bacillus anthracis* toxin. *Infect. Immun.* 45:761-767.
7. Friedlander, A. M. 1986. Macrophages are sensitive to anthrax lethal toxin through an acid-dependent process. *J. Biol. Chem.* 261:7123-7126.
8. Klimpel, K. R., S. S. Molloy, G. Thomas, and S. H. Leppla. 1992. Anthrax toxin protective antigen is activated by a cell surface protease with the sequence specificity and catalytic properties of furin. *Proc. Natl. Acad. Sci. USA* 89:10277-10281.
9. Little, S. F., and G. B. Knudson. 1986. Comparative efficacy of *Bacillus anthracis* live spore vaccine and protective antigen vaccine against anthrax in the guinea pig. *Infect. Immun.* 52:509-512.
10. Little, S. F., J. M. Novak, R. J. Lowe, S. H. Leppla, Y. Singh, K. R. Klimpel, B. C. Lidgerding, and A. M. Friedlander. 1996. Characterization of lethal factor binding and cell receptor binding domains of protective antigen of *Bacillus anthracis* using monoclonal antibodies. *Microbiology* 142:707-715.
11. McBride, B. W., A. Mogg, J. L. Telfer, M. S. Lever, J. Miller, P. C. B. Turnbull, and L. Baillie. 1998. Protective efficacy of a recombinant protective antigen against *Bacillus anthracis* challenge and assessment of immunological markers. *Vaccine* 16:810-818.
12. Miller, J., B. W. McBride, R. J. Manchec, P. Moore, and L. W. J. Baillie. 1998. Production and purification of recombinant protective antigen and protective efficacy against *Bacillus anthracis*. *Lett. Appl. Microbiol.* 26:56-60.
13. Milne, J. C., D. Furlong, P. C. Hanna, J. S. Wall, and R. J. Collier. 1994. Anthrax protective antigen forms oligomers during intoxication of mammalian cells. *J. Biol. Chem.* 269:20607-20612.
14. Mogridge, J., M. Mourez, and R. J. Collier. 2001. Involvement of domain 3 in oligomerization by the protective antigen moiety of anthrax toxin. *J. Bacteriol.* 183:2111-2116.
15. Petosa, C., R. J. Collier, K. R. Klimpel, S. H. Leppla, and R. C. Liddington. 1997. Crystal structure of the anthrax toxin protective antigen. *Nature* 385:833-838.
16. Quinn, C. P., and P. C. B. Turnbull. 1998. Anthrax, p. 799-818. In L. Collier, A. Barlow, and M. Sussman (ed.), *Topley and Wilson's microbiology and microbial infections*. Arnold, London, United Kingdom.
17. Turnbull, P. C. B. 1991. Anthrax vaccines: past, present and future. *Vaccine* 9:533-539.
18. Turnbull, P. C. B., M. G. Broster, J. A. Carman, R. J. Manchec, and J. Melling. 1986. Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance to protective immunity. *Infect. Immun.* 52:356-363.
19. Varughese, M., A. V. Teixeira, S. Liu, and S. H. Leppla. 1999. Identification of a receptor-binding region within domain 4 of the protective antigen component of anthrax toxin. *Infect. Immun.* 67:1860-1865.
20. Williamson, E. D., R. J. Beedham, A. M. Bennet, S. D. Perkins, J. Miller, and L. W. J. Baillie. 1999. Presentation of protective antigen to the mouse immune system: immune sequelae. *J. Appl. Microbiol.* 87:315-317.

Editor: J. D. Clements